

## Thermo Scientific GeneJET Plasmid Miniprep Kit

- All purification steps should be carried out at room temperature.
- All centrifugations should be carried out in a table-top microcentrifuge at  $>12000 \times g$  (10 000-14 000 rpm, depending on the rotor type).

1. Resuspend the pelleted cells in 250  $\mu\text{L}$  of the Resuspension Solution. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. Add 250  $\mu\text{L}$  of the Lysis Solution and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear.

3. Add 350  $\mu\text{L}$  of the Neutralization Solution and mix immediately and thoroughly by inverting the tube 4-6 times.

4. Centrifuge for 5 min to pellet cell debris and chromosomal DNA.

5. Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate.

6. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.

7. Add 500  $\mu\text{L}$  of the Wash Solution to the GeneJET spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.

8. Repeat the wash procedure (step 7) using 500  $\mu\text{L}$  of the Wash Solution.

9. Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.

10. Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube (not included). Add 50  $\mu\text{L}$  of the Elution Buffer to the center of GeneJET spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min.

11. Discard the column and store the purified plasmid DNA at  $-20^{\circ}\text{C}$ .

From: Thermo Scientific